

WHAT IS CLAIMED IS:

1. A substantially purified single chain polypeptide, comprising the protease domain of a type-II membrane-type serine protease (MTSP) or a catalytically active portion thereof, wherein:
 - 5 the MTSP portion of the protein consists essentially of the protease domain of the MTSP or a catalytically active portion thereof.
 2. The substantially purified polypeptide of claim 1, wherein the MTSP is not expressed endothelial cells.
 3. The substantially purified polypeptide of claim 1, wherein the 10 MTSP is not expressed on normal endothelial cells *in vivo*.
 4. The substantially purified polypeptide of claim 1, wherein the MTSP is a human protein.
 5. The substantially purified polypeptide of claim 1 that consists essentially of the protease domain of an MTSP or a catalytically active 15 portion of the protease domain.
 6. The substantially purified polypeptide of claim 1, wherein the expression and/or activity of the MTSP in tumor cells differs from its level of expression and/or activity in non-tumor cells.
 7. The substantially purified polypeptide of claim 1, wherein the 20 MTSP is detectable in a body fluid at a level that differs from its level in body fluids in a subject not having a tumor.
 8. The substantially purified polypeptide of claim 1, wherein:
the MTSP is present in a tumor; and
a substrate or cofactor for the MTSP is expressed at levels that 25 differ from a non-tumor cell in the same tissue.
 9. The substantially purified polypeptide of claim 1, wherein:
the MTSP exhibits altered substrate specificity in the tumor compared to its specificity in a non-tumor cell in the same tissue.
 10. The substantially purified polypeptide of claim 1, wherein the 30 MTSP has an N-terminus that comprises IVNG, ILGG, VGLL or ILGG.

11. The substantially purified polypeptide of claim 1, wherein the MTSP is selected from among MTSP1, MTSP3, MTSP4 and MTSP6.

12. The substantially purified polypeptide of claim 1, wherein the protease domain comprises the sequence of amino acids set forth as

5 amino acids 615-855 of SEQ ID No. 2, as amino acids 205-437 of SEQ ID NO. 4, as the amino acids in SEQ ID No. 6, or as amino acids 217-443 in SEQ ID No. 12.

13. The substantially purified polypeptide of claim 1 that has at least about 40%, 60%, 80%, 90% or 95% sequence identity with a

10 protease domain that comprises the sequence of amino acids set forth as amino acids 615-855 of SEQ ID No. 2, as amino acids 205-437 of SEQ ID NO. 4, as the amino acids in SEQ ID No. 6, or as amino acids 217-443 in SEQ ID No. 12.

14. A polypeptide of claim 1, wherein the protease domain
15 portion is encoded by a nucleic acid molecule that hybridizes under conditions of high stringency along its full length to a nucleic acid molecule comprising a sequence of nucleotides set forth in SEQ ID No: 1, 3, 5, 7, 9 or 11 or to a molecule that encodes the protein set forth in SEQ ID No: 2, 4, 6, 8, 10 or 12 or at least one domain thereof.

20 15. A nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of claim 1.

16. A mutein of the polypeptide of claim 1, wherein:
up to about 90% of the amino acids are replaced with another amino acid;

25 and the resulting polypeptide is a single chain and has catalytic activity at least 10% of the unmutated polypeptide.

17. The mutein of claim 16, wherein up to about 95% of the amino acids are replaced.

18. The mutein of claim 16, wherein the resulting polypeptide is
30 a single chain and has catalytic activity at least 50% of the unmutated polypeptide.

19. A mutein of the polypeptide of claim 1, wherein a free Cys in the protease domain is replaced with another amino acid, whereby the resulting polypeptide exhibits proteolytic activity.

20. A mutein of the polypeptide of claim 1, wherein a free Cys
5 in the protease domain is replaced with a serine.

21. A vector comprising the nucleic acid molecule of claim 15.

22. The vector of claim 21 that is an expression vector.

23. The vector of claim 21 that includes a sequence of nucleotides that directs secretion of any protein encoded by a sequence
10 of nucleotides operatively linked thereto.

24. The vector of claim 21 that is a *Pichia* vector or an *E. coli* vector.

25. A cell, comprising the vector of claim 21.

26. The cell of claim 25 that is a prokaryotic cell.

15 27. The cells of claim 25 that is a eukaryotic cell.

28. The cell of claim 25 that is selected from among a bacterial cell, a yeast cell, a yeast cell, a plant cell, an insect cell and an animal cell.

29. The cell of claim 25 that is a mammalian cell.

20 30. A method for producing a polypeptide that contains a protease domain of an MTSP, comprising:
culturing the cell of claim 25 under conditions whereby the encoded protein is expressed by the cell; and
recovering the expressed protein.

25 31. The method of claim 30, wherein the cell is a pichia cell and the protein is secreted into the culture medium.

32 An antisense nucleic acid molecule that comprises at least
14 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the protease
30 domain of an MTSP of claim 1; or

comprises at least 16 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the protease domain of an MTSP of claim 1.

33. An antibody that specifically binds to the single chain form
 5 of a protease domain of the polypeptide of claim 1, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

34. The polypeptide of claim 1, wherein the MTSP is selected from among corin, MTSP1, enterpeptidase, human airway trypsin-like
 10 protease (HAT), MTSP1, TMPRSS2, and TMPRSS4.

35. A conjugate, comprising:
 a) a protein of claim 1, and
 b) a targeting agent linked to the protein directly or via a linker.
 36. The conjugate of claim 35, wherein the targeting agent
 15 permits
 i) affinity isolation or purification of the conjugate;
 ii) attachment of the conjugate to a surface;
 iii) detection of the conjugate; or
 37 iv) targeted delivery to a selected tissue or cell.

20 ~~36.~~ A combination, comprising:
 a) an inhibitor of the catalytic activity of the polypeptide of
 claim 1; and
 b) another treatment or agent selected from anti-tumor and
 38 anti-angiogenic treatments or agents.

25 ~~37.~~ The combination of claim 36, wherein the inhibitor and the
 anti-tumor and/or anti-angiogenic agent are formulated in a single
 pharmaceutical composition or each is formulated in separate
 pharmaceutical compositions.

39 ~~38.~~ The combination of claim 36, wherein the inhibitor is
 30 selected from antibodies and antisense oligonucleotides.

39. A solid support comprising two or more polypeptides of claim 1 linked thereto either directly or via a linker.

40. The support of claim 39, wherein the polypeptides comprise an array.

5 41. The support of claim 39, wherein the polypeptides comprise a plurality of different MTSP protease domains.

42. A method for identifying compounds that modulate the protease activity of an MTSP, comprising:

contacting a polypeptide of claim 1 with a substrate proteolytically 10 cleaved by the MTSP, and, either simultaneously, before or after, adding a test compound or plurality thereof;

measuring the amount of substrate cleaved in the presence of the test compound; and

15 selecting compounds that change the amount cleaved compared to a control, whereby compounds that modulate the activity of the MTSP are identified.

43. The method of claim 42, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof.

20 44. The method of claim 42, wherein a plurality of the test substances are screened simultaneously.

45. The method of claim 42, wherein the change in the amount cleaved is assessed by comparing the amount cleaved in the presence of the test compound with the amount in the absence of the test compound.

25 46. The method of claim 42, wherein a plurality of the test substances are screened for simultaneously.

47. The method of claim 42, wherein a plurality of the polypeptides are linked to a solid support, either directly or via a linker.

30 48. The method of claim 42, wherein the polypeptides comprise an array.

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 49. The method of claim 42, wherein the polypeptides comprise a plurality of different MTSP proteases.

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 50. A method of identifying a compound that specifically binds to a single chain protease domain of an MTSP, comprising:

- 5 contacting a polypeptide of claim 1 with a test compound or plurality thereof under conditions conducive to binding thereof; and identifying compounds that specifically bind to the MTSP single chain protease domain or compounds that inhibit binding of a compound known to bind to the MTSP single chain protease domain, wherein the
- 10 known compound is contacted with the polypeptide before, simultaneously with or after the test compound.

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 51. The method of claims 50, wherein the polypeptide is linked either directly or indirectly via a linker to a solid support.

- 15 52. The method of claim 50, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof.

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 53. The method of claim 50, wherein a plurality of the test substances are screened for simultaneously.

- 20 54. The method of claim 51, wherein a plurality of the polypeptides are linked to a solid support.

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 55. A substantially purified membrane-type serine protease 3 (MTSP3).

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 56. The MTSP3 of claim 43 that is selected from the group consisting of:

- 25 a polypeptide encoded by the sequence of nucleotides set forth in SEQ ID No. 3;
- a polypeptide encoded by a sequence of nucleotides that hybridizes under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 3;
- 30 a polypeptide that comprises the sequence of amino acids set forth as amino acids 205-437 of SEQ ID No. 4;

a polypeptide that comprises a sequence of amino acids having at least about 90% sequence identity with the sequence of amino acids set forth in SEQ ID No. 4; and

5 a polypeptide encoded by a splice variant of the sequence of nucleotides set forth in SEQ ID No. 3.

57. A nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of claim 56.

58. A vector comprising the nucleic acid molecule of claim 57.

60 59. A cell, comprising the vector of claim 58.

10 60. The cell of claim 59 that expresses the MTSP3 on its surface.

61. The cell of claim 59 that is a prokaryotic cell.

62. The cells of claim 59 that is a eukaryotic cell.

63. The cell of claim 59 that is selected from among a bacterial 15 cell, a yeast cell, a yeast cell, a plant cell, an insect cell and an animal cell.

64. The cell of claim 59 that is a mammalian cell.

65. The cell of claim 59, wherein the MTSP3 comprises the sequence of amino acids set forth in SEQ ID No. 4; or a sequence of 20 amino acids encoded by a sequence of nucleotides that hybridizes under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 3; or a protein having at least about 90% sequence identity with the sequence of amino acids set forth in SEQ ID No. 4 and retaining protease activity.

25 66. A method for producing an MTSP3, comprising:

culturing the cell of claim 59 under conditions whereby the encoded protein is expressed by the cell; and

recovering the expressed protein.

67. An antisense nucleic acid molecule that comprises at least 30 14 contiguous nucleotides or modified nucleotides that are

complementary to a contiguous sequence of nucleotides in the nucleic acid molecule of claim 57; or

comprises at least 16 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of

5 nucleotides in the in the nucleic acid molecule of claim 57.

68. An antibody that specifically binds to the MTSP of claim 57, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

10 69. A method for treating or preventing a neoplastic disease, in a mammal, comprising administering to a mammal an effective amount of an inhibitor of an MTSP3 of claim 55.

70. The method of claim 69, wherein the inhibitor is an antibody that specifically binds to the MTSP3, or a fragment or derivative of the 15 antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

71. A substantially purified membrane-type serine protease 4 (MTSP4).

72. The substantially purified MTSP4 that is an MTSP4-L or an 20 MTSP4-S.

73. The MTSP4 of claim 71 that is selected from the group consisting of:

a polypeptide encoded by the sequence of nucleotides set forth in SEQ ID No. 7 or 9;

25 a polypeptide encoded by a sequence of nucleotides that hybridizes under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 7 or 9;

a polypeptide that comprises the sequence of amino acids set forth in SEQ ID No. 6, 8 or 10; and

30 a polypeptide encoded by a splice variant of the sequence of nucleotides set forth in SEQ ID No. 7 or 9.

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74. The MTSP4 of claim 73 that is an MTSP4-L or an MTSP4-S.

75. A nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of claim 73.

76. A vector comprising the nucleic acid molecule of claim 74.

5 77. A cell, comprising the vector of claim 76.

78. The cell of claim 77 that expresses the MTSP4 on its surface.

79. The cell of claim 77 that is a prokaryotic cell.

80. The cells of claim 77 that is a eukaryotic cell.

10 81. The cell of claim 77 that is selected from among a bacterial cell, a yeast cell, a yeast cell, a plant cell, an insect cell and an animal cell.

82. The cell of claim 77 that is a mammalian cell.

83. The cell of claim 77, wherein the MTSP4 comprises the

15 sequence of amino acids set forth in SEQ ID No. 6, 8 or 10; or a sequence of amino acids encoded by a sequence of nucleotides that hybridizes under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 7 or 9; or a protein having at least about 90% sequence identity with the sequence of amino acids set forth

20 in SEQ ID No. 8 or 10 and retaining protease activity.

84. A method for producing an MTSP4, comprising:
culturing the cell of claim 77 under conditions whereby the encoded protein is expressed by the cell; and
recovering the expressed protein.

25 85. An antisense nucleic acid molecule that comprises at least 14 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the nucleic acid molecule of claim 75 or
comprises at least 16 contiguous nucleotides or modified

30 nucleotides that are complementary to a contiguous sequence of nucleotides in the nucleic acid molecule of claim 75.

86. An antibody that specifically binds to the MTSP of claim 72, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

5 87. An antibody that specifically binds to the MTSP of claim 73, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

10 88. A method for treating or preventing a neoplastic disease, in a mammal, comprising administering to a mammal an effective amount of an inhibitor of an MTSP4 of claim 71.

15 89. The method of claim 88, wherein the inhibitor is an antibody that specifically binds to an MTSP4, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

90. A substantially purified membrane-type serine protease 6 (MTSP6) selected from the group consisting of:

a polypeptide encoded by the sequence of nucleotides set forth in SEQ ID No. 11;

20 a polypeptide encoded by a sequence of nucleotides that hybridizes along the full length thereof under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 11;

a polypeptide that comprises the sequence of amino acids set forth as amino acids 217-443 of SEQ ID No. 12;

25 a polypeptide encoded by a splice variant of the sequence of nucleotides set forth in SEQ ID No. 11.

91. A nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of claim 90.

92. A vector comprising the nucleic acid molecule of claim 91.

30 93. A cell, comprising the vector of claim 92.

94. The cell of claim 93 that expresses the MTSP6 on its surface.

95. The cell of claim 93 that is a prokaryotic cell.

96. The cells of claim 93 that is a eukaryotic cell.

5 97. The cell of claim 93 that is selected from among a bacterial cell, a yeast cell, a yeast cell, a plant cell, an insect cell and an animal cell.

98. The cell of claim 93 that is a mammalian cell.

99. The cell of claim 93, wherein the MTSP6 comprises the

10 sequence of amino acids set forth in SEQ ID No. 12; or a sequence of amino acids encoded by a sequence of nucleotides that hybridizes along the full length thereof under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 12; or a protein having at least about 95% sequence identity with the sequence of amino acids set forth

15 in SEQ ID No. 4 and retaining protease activity.

100. A method for producing an MTSP6, comprising:
culturing the cell of claim 93 under conditions whereby the
encoded protein is expressed by the cell; and
recovering the expressed protein.

20 101. An antisense nucleic acid molecule that comprises at least 14 contiguous nucleotides or modified nucleotides that are complementary to a contiguous sequence of nucleotides in the nucleic acid molecule of claim 91; or
comprises at least 16 contiguous nucleotides or modified

25 nucleotides that are complementary to a contiguous sequence of nucleotides in the nucleic acid molecule of claim 91.

102. An antibody that specifically binds to the MTSP of claim 90, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal

30 antibody.

103. A method for treating or preventing a neoplastic disease, in a mammal, comprising administering to a mammal an effective amount of an inhibitor of an MTSP6 of claim 90.

104. The method of claim 103, wherein the inhibitor is an antibody that specifically binds to the MTSP6, or a fragment or derivative of the antibody containing a binding domain thereof, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

105. A recombinant non-human animal, wherein an endogenous gene of an MTSP has been deleted or inactivated by homologous recombination or insertional mutagenesis of the animal or an ancestor thereof.

106. A recombinant non-human animal of claim 105, wherein the MTSP is an MTSP1, MTSP3, MTSP4 or MTSP6.

107. A conjugate, comprising:

15 a) an MTSP3 or MTSP4 or an MTSP6 of claim 90; and
 b) a targeting agent linked to the protein directly or via a linker.

108. The conjugate of claim 106, wherein the targeting agent permits

20 i) affinity isolation or purification of the conjugate;
 ii) attachment of the conjugate to a surface;
 iii) detection of the conjugate; or
 iv) targeted delivery to a selected tissue or cell.

109. A combination, comprising:

25 a) an inhibitor of the catalytic activity of an MTSP3 or MTSP4 or MTSP6 of claim 90; and
 b) another treatment or agent selected from anti-tumor and anti-angiogenic treatments or agents.

110. The combination of claim 109, wherein the inhibitor and the anti-tumor and/or anti-angiogenic agent are formulated in a single pharmaceutical composition or each is formulated in separate pharmaceutical compositions.

111. The combination of claim 109, wherein the inhibitor is selected from antibodies and antisense oligonucleotides.
112. A solid support comprising two or more MTSP3 polypeptides and/or MTSP4 polypeptides and/or MTSP6 polypeptides of claim 90 linked thereto either directly or via a linker.
 - 5 113. The support of claim 112, wherein the polypeptides comprise an array.
 114. A method for identifying compounds that modulate the protease activity of an MTSP selected from MTSP3, MTSP4 or MTSP6 of claim 90, comprising:
 - 10 contacting the MTSP with a substrate proteolytically cleaved by the MTSP, and, either simultaneously, before or after, adding a test compound or plurality thereof;
 - 15 measuring the amount of substrate cleaved in the presence of the test compound; and
 - selecting compounds that change the amount cleaved compared to a control, whereby compounds that modulate the activity of the MTSP are identified.
 115. The method of claim 114, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof.
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 116. The method of claim 114, wherein a plurality of the test substances are screened for simultaneously.
 117. The method of claim 114, wherein the change in the amount cleaved is assessed by comparing the amount cleaved in the presence of the test compound with the amount in the absence of the test compound.
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 118. The method of claim 114, wherein a plurality of the test substances are screened for simultaneously.
 119. The method of claim 118, wherein a plurality of the polypeptides are linked to a solid support.
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120. A method of identifying a compound that specifically binds to a MTSP selected from MTSP3, MTSP4 and the MTSP6 of claim 90, comprising:

5 contacting the MTSP polypeptide with a test compound or plurality thereof under conditions conducive to binding thereof; and identifying compounds that specifically binds to the MTSP.

121. A method of identifying a compound that specifically binds to a MTSP selected from MTSP3, MTSP4 and the MTSP6 of claim 90, comprising:

10 contacting the MTSP polypeptide with a test compound or plurality thereof under conditions conducive to binding thereof; and identifying compounds that specifically binds to the MTSP.

122. The method of any claims 121, wherein the polypeptide is linked either directly or indirectly via a linker to a solid support.

15 123. The method of claim 121, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof.

124. The method of claim 121, wherein a plurality of the test substances are screened for simultaneously.

20 125. The method of claim 124, wherein a plurality of the polypeptides are linked to a solid support.

126. An MTSP6 polypeptide, comprising amino acids set forth as amino acids 46-55 in SEQ ID No. 12 and/or amino acids 368-394 of SEQ ID No. 12, and that is encoded by a sequence of nucleic acids that
25 hybridizes under moderate stringency to nucleic acid encoding the polypeptide set forth in SEQ ID No. 12.

127. The polypeptide of claim 126 that comprises the amino acids set forth as amino acids 46-55 in SEQ ID No. 12 and/or amino acids 368-394 of SEQ ID No. 12, and that is encoded by a sequence of nucleic acids that hybridizes under high stringency along its full length or full
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length of the protease domain to nucleic acid encoding the polypeptide set forth in SEQ ID No. 12.

128. The polypeptide of claim 126, comprising the sequence of amino acids set forth in SEQ ID No. 12.

5 129. An isolated nucleic acid molecule, comprising a sequence of nucleic acids that encodes the polypeptide of claim 126.

10 130. A method for treating tumors, comprising administering a prodrug that is specifically cleaved by an MTSP, whereby, upon contact with a cell that expresses MTSP activity, the prodrug is converted into an active drug.

131. The method of claim 130, wherein the MTSP is selected from among an MTSP3, MTSP4 and an MTSP6 of claim 90.

15 132. The method of claim 130, wherein the MTSP is selected from among corin, enterpeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4.

133. A method of detecting neoplastic disease, comprising: detecting an MTSP3, MTSP4 or MTSP6 of claim 90 in a biological sample, wherein the amount detected differs from the amount in a subject who does not have neoplastic disease.

20 134. The method of claim 133, wherein the biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, cerebrospinal fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

25 135. The method of claim 133, wherein the extracellular domain of the MTSP3, MTSP4 or MTSP6 is in the sample.